OFFICE OF HUMAN RESEARCH ETHICS
Institutional Review Board

APPLICATION FOR IRB APPROVAL OF
HUMAN SUBJECTS RESEARCH
Version 19-Feb-2008

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Part A.1. Contact Information, Agreements, and Signatures

Date: March 3, 2008

Title of Study: Cardioprotective Effects of Omega-3 Fatty Acids Supplementation in Healthy Older Subjects Exposed to Diesel Exhaust

Name and degrees of Principal Investigator: Haiyan Tong, MD, PhD; James M Samet, PhD, MPH
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For trainee-led projects: __ undergraduate __ graduate _X_ postdoc __ resident __ other
Name of faculty advisor: James M Samet, PhD, MPH
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Center, institute, or department in which research is based if other than department(s) listed above:

Name of Project Manager or Study Coordinator (if any):
Department: Mailing address/CB #:
Phone #: Fax #: Email Address:

List **all other project personnel** including co-investigators, and anyone else who has contact with subjects or identifiable data from subjects. **Include email address for each person who should receive electronic copies of IRB correspondence to PI:** Martha Almond RRT; Maryann Bassett, RN; Philip Bromberg, MD; Martha Sue Carraway, MD; Martin Case, BS; Wayne Cascio, MD; Melissa Caughey, BS, RVT; David DeMarini, PhD; Andrew Ghio MD; Milan Hazucha, MD, PhD; Alan Hinderliter, MD; Fernando Holguin, MD; Debbie Levin, BSN; Margaret Herbst, RN, MSN; Sally Ivins, BA; Howard Kehrl, MD; Tracey Montilla, RN; Lynne Newlin-Clapp, BA; Dave Peden, MD, MS; Carole Robinette, MS; Michael Schmitt, MS; and Susan Steck, PhD, RD; Haibo Zhou, PhD.

Name of funding source or sponsor (*please do not abbreviate*):

__ not funded _X_ Federal __ State __ industry __ foundation __ UNC-CH

__ other (specify): EPA Intramural Federal Research

For industry sponsored research (if applicable):

Sponsor's master protocol version #: Version date:

Investigator Brochure version #: Version date:

Any other details you need documented on IRB approval:

RAMSeS proposal number (from Office of Sponsored Research):

Checklist of Items to Include with Your Submission

Include the following items with your submission, where applicable.

- Check the relevant items below and include one copy of all checked items 1-11 in the order listed.
- Also include two additional collated sets of copies (sorted in the order listed) for items 1-7.

→ **Applications will be returned if these instructions are not followed.**

Check	Item	Total No. of Copies
X	1. This application. One copy must have original PI signatures.	3
□	2. Consent and assent forms, fact or information sheets; include phone and verbal consent scripts.	3
□	3. HIPAA authorization addendum to consent form.	3
□	4. All recruitment materials including scripts, flyers and advertising, letters, emails.	3
□	5. Questionnaires, focus group guides, scripts used to guide phone or in-person interviews, etc.	3
□	6. Documentation of reviews from any other committees (e.g., GCRC, Oncology Protocol Review Committee, or local review committees in Academic Affairs).	3
□	7. Protocol, grant application or proposal supporting this submission, if any (e.g., extramural grant application to NIH or foundation, industry protocol, student proposal). This must be submitted if an external funding source or sponsor is checked on the previous page.	1
□	8. Addendum for Multi-Site Studies where UNC-CH is the Lead Coordinating Center.	1
□	9. Data use agreements (may be required for use of existing data from third parties).	1
□	10. Only for those study personnel *not* in the online UNC-CH human research ethics training database (http://cfx3.research.unc.edu/training_comp/): Documentation of required training in human research ethics.	1
□	11. Investigator Brochure if a drug study.	1

Principal Investigator: I will personally conduct or supervise this research study. I will ensure that this study is performed in compliance with all applicable laws, regulations and University policies regarding human subjects research. I will obtain IRB approval before making any changes or additions to the project. I will notify the IRB of any other changes in the information provided in this application. I will provide progress reports to the IRB at least annually, or as requested. I will report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. I will follow the IRB approved consent process for all subjects. I will ensure that all collaborators, students and employees assisting in this research study are informed about these obligations. All information given in this form is accurate and complete.

Signature of Principal Investigator — Date: 3/3/08

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that this study complies with all the obligations listed above for the PI.

Signature of Faculty Advisor — Date: 3/3/08

Note: The following signature is not required for applications with a student PI.

Department or Division Chair, Center Director (or counterpart) of PI: (or Vice-Chair or Chair's designee if Chair is investigator or otherwise unable to review): I certify that this research is appropriate for this Principal Investigator, that the investigators are qualified to conduct the research, and that there are adequate resources (including financial, support and facilities) available. If my unit has a local review committee for pre-IRB review, this requirement has been satisfied. I support this application, and hereby submit it for further review.

Signature of Department Chair or designee — Date

Print Name of Department Chair or designee — Department

Part A.2. Summary Checklist *Are the following involved?*	Yes	No
A.2.1. Existing data, research records, patient records, and/or human biological specimens?	__	_X_
A.2.2. Surveys, questionnaires, interviews, or focus groups with subjects?	_X_	__
A.2.3. Videotaping, audiotaping, filming of subjects, or analysis of existing tapes?	__	_X_
A.2.4. Do you plan to enroll subjects from these vulnerable or select populations: a. UNC-CH students or UNC-CH employees? b. Non-English-speaking? c. Decisionally impaired? d. Patients? e. Prisoners, others involuntarily detained or incarcerated, or parolees? f. Pregnant women? g. Minors (less than 18 years)? ***If yes***, give age range: to years	 _X_ __ __ _X_ __ __ __	 __ _X_ X__ __ _X_ _X_ _X_
A.2.5. a. Are sites outside UNC-CH engaged in the research? b. Is UNC-CH the sponsor or lead coordinating center for a multi-site study? ***If yes***, include the ***Addendum for Multi-site Studies***. ***If yes***, will any of these sites be outside the United States? ***If yes***, is there a local ethics review committee agency with jurisdiction? (provide contact information)	__ __ __ __	_X_ _X_ __ __
A.2.6. Will this study use a data and safety monitoring board or committee? ***If yes:*** UNC-CH School of Medicine DSMB? (must apply separately) Lineberger Cancer Center DSMC? Other? Specify:	__ __ __ __	_X_ __ __ __
A.2.7. a. Are you collecting sensitive information such as sexual behavior, HIV status, recreational drug use, illegal behaviors, child/physical abuse, immigration status, etc? b. Do you plan to obtain a federal Certificate of Confidentiality for this study?	__ __	_X_ _X_
A.2.8. a. Investigational drugs? (provide **IND #**) b. Approved drugs for "non-FDA-approved" conditions? *All studies testing substances in humans must provide a letter of acknowledgement from the UNC Health Care Investigational Drug Service (IDS).*	__ __	_X_ _X_
A.2.9. Placebo(s)?	__	_X_
A.2.10. Investigational devices, instruments, machines, software? (provide **IDE #**)	__	_X_
A.2.11. Fetal tissue?	__	_X_
A.2.12. Genetic studies on subjects' specimens?	_X_	__
A.2.13. Storage of subjects' specimens for future research? ***If yes, see instructions for Consent for Stored Samples.***	_X_	__
A.2.14. Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise? ***If yes, approval by the UNC-CH Radiation Safety Committee is required.***	__	_X_
A.2.15. Recombinant DNA or gene transfer to human subjects? ***If yes, approval by the UNC-CH Institutional Biosafety Committee is required.***	__	_X_
A.2.16. Does this study involve UNC-CH cancer patients? ***If yes, submit this application directly to the Oncology Protocol Review Committee.***	__	_X_
A.2.17. Will subjects be studied in the General Clinical Research Center (GCRC)? ***If yes, obtain the GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.***	_X_	__
A.2.18. Will gadolinium be administered as a contrast agent?	__	_X_

Part A.3. Conflict of Interest Questions and Certification

The following questions apply to **all investigators and study staff** engaged in the design, conduct, or reporting results of this project **and/or their immediate family members.** For these purposes, "family" includes the individual's spouse and dependent children. "Spouse" includes a person with whom one lives together in the same residence and with whom one shares responsibility for each other's welfare and shares financial obligations.

A.3.1. Currently or during the term of this research study, does any member of the research team or his/her family member have or expect to have:		
(a) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with the sponsor of this study?	__ yes	_X no
(b) A personal financial interest in or personal financial relationship (including gifts of cash or in-kind) with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no
(c) A board membership of any kind or an executive position (paid or unpaid) with the sponsor of this study or with an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no
A.3.2. Has the University or has a University-related foundation received a cash or in-kind gift from the sponsor of this study for the use or benefit of any member of the research team?	__ yes	_X no
A.3.3. Has the University or has a University-related foundation received a cash or in-kind gift for the use or benefit of any member of the research team from an entity that owns or has the right to commercialize a product, process or technology studied in this project?	__ yes	_X no

If the answer to ANY of the questions above is *yes*, the affected research team member(s) must complete and submit to the Office of the University Counsel the form accessible at http://coi.unc.edu. List name(s) of all research team members for whom any answer to the questions above is *yes*:

_____

Certification by Principal Investigator: By submitting this IRB application, I (the PI) certify that the information provided above is true and accurate regarding my own circumstances, that I have inquired of every UNC-Chapel Hill employee or trainee who will be engaged in the design, conduct or reporting of results of this project as to the questions set out above, and that I have instructed any such person who has answered "yes" to any of these questions to complete and submit for approval a Conflict of Interest Evaluation Form. I understand that as Principal Investigator I am obligated to ensure that any potential conflicts of interest that exist in relation to my study are reported as required by University policy.

_____ _____

Signature of Principal Investigator Date

Faculty Advisor if PI is a Student or Trainee Investigator: I accept ultimate responsibility for ensuring that the PI complies with the University's conflict of interest policies and procedures.

_____ _____

Signature of Faculty Advisor Date

Part A.4. Questions Common to All Studies

For all questions, if the study involves only secondary data analysis, focus on your proposed design, methods and procedures, and not those of the original study that produced the data you plan to use.

A.4.1. **Brief Summary**. Provide a *brief* non-technical description of the study, which will be used in IRB documentation as a description of the study. Typical summaries are 50-100 words. *Please reply to each item below, retaining the subheading labels already in place, so that reviewers can readily identify the content.*

Purpose: A growing body of epidemiological data suggests an increased risk of cardiovascular events associated with air pollutants. Reactive oxygen species (ROS) have been implicated as a potential mechanism for the adverse effects of air pollutants and genetic polymorphisms of the glutathione-s-transferases (GSTs) have been shown to participate in the antioxidant defenses to air pollutants. The first part of this protocol is a pilot study to identify the optimum diesel exhaust concentration to investigate the cardiovascular effects in healthy older subjects. The main part of this proposal is to examine the health effects of diesel exhaust exposure on the cardiovascular system and to examine whether omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We will also determine whether healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust.
Participants: Approximately 6 healthy 50-75 year-old male and female subjects with both GSTM1 null and GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val; n=2 each) for the pilot study. Sixty healthy 50-75 year-old male and female subjects (30 subjects are GSTM1 positive genotype and 30 subjects are GSTM1 null) for the main study.
Procedures (methods): In the pilot study, subjects will have 3 sequential exposures to the diesel exhausts at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours with a about 2 weeks of interval between exposures. In the main study, GSTM1 positive and GSTM1 null genotype subjects will be exposed to the optimum concentration (will be determined based on the pilot study) of diesel exhaust for 2 hours after 15 days of omega-3 fatty acid fish oil or olive oil supplementations.

A.4.2. **Purpose and Rationale**. Provide a summary of the background information, state the research question(s), and tell why the study is needed. If a complete rationale and literature review are in an accompanying grant application or other type of proposal, only provide a brief summary here. If there is no proposal, provide a more extensive rationale and literature review, including references.

Numerous epidemiological studies have demonstrated an association between acute and chronic exposure to different levels of air pollution and various adverse cardiopulmonary effects including mortality, respiratory tract infection, exacerbation of asthma, chronic bronchitis, ischemic heart disease, and stroke (see review, (1)). A recent national scale epidemiological study has shown that short-term exposure to particulate matter (PM) is associated with increased rates of hospital admission for cardiovascular and respiratory symptoms. The cardiovascular risk tended to be higher in the Eastern United States. This study also indicated an ongoing threat to the health of the elderly population from air-borne particles (2). Although air pollution exposure has long been known to be a risk factor for respiratory disease, over the last decade, a growing body of epidemiological studies has heightened concern about the increased risk of cardiovascular events related to both short-term and long-term exposure to air pollutants (3). The risk of death from

cardiovascular disease (myocardial infraction, heart failure, and fatal arrhythmias) in response to chronically high levels of air pollution was much greater than that from lung disease (4-6). Short-term elevations in ambient PM levels are capable of evoking cardiac arrhythmias, worsening heart failure, and triggering acute atherosclerotic/ischemic cardiovascular complications, particularly in certain at-risk subsets of population (3). PM exposure can result in increases in heart rate, and decreases in heart rate variability (HRV; defined as changes in mean heart rate during 24 hrs which is a reflection of the cardiac autonomic function) by depression the cardiac autonomic nervous system (7). PM has been associated with transient increases in plasma viscosity (8), endothelial dysfunction (9), acute-phase reactants (10, 11), and C-reactive protein (12). Animal studies have suggested that long-term exposure to low concentration of PM altered vasomotor tone, induces vascular inflammation and potentates atherosclerosis (13).

A recent epidemiological study involving eight European cities suggested that the primary effect of PM air pollution on cardiac admissions was likely attributable to diesel exhaust inhalation (14). Diesel exhaust is a component of air pollution in urban settings, and contributes both PM and gaseous phase compounds to the atmosphere. Diesel exhaust fumes are made up largely of fine and ultrafine carbonaceous particulates generated by incomplete combustion of fuel. The U.S. EPA and the National Research Council (NRC) PM Research Priorities currently include identification of components of PM (including PM size and chemical composition) that contribute to the reported increased morbidity and mortality (NRC, 1998; EPA, 1997). In addition, both the EPA and NRC have stressed the importance of examining gaseous co-pollutants that may also affect PM toxicity. Diesel exhaust (i.e., gas phase constituents and diesel exhaust particles (DEP)) is ideal for examining the role of these factors in inducing human health effects for the following reasons: (1) Because of the relatively high content of organic compounds on DEP (usually 10-30% by weight), DEP are ideal for examining the role of organics in inducing health effects; (2) The exhaust is a mixture of PM and gaseous pollutants including some (e.g., aldehydes, NOx, carbon monoxide) that are known to produce human toxicity in high enough concentrations that may potentially augment PM-induced effects; (3) the size of the DEP vary depending on engine type and other factors, allowing assessment of PM size on the induction of human health effects. Few controlled human exposures to diesel exhaust have been performed. One hour exposure to 300 $\mu g/m^3$ diesel exhaust with intermittent exercise in healthy human volunteers resulted in marked systemic and redox-sensitive pulmonary inflammatory responses (15, 16), and vascular dysfunction and impaired endogenous fibrinolysis that links diesel exhaust inhalation to the pathogenesis of atherothrombosis and acute myocardial infarction (17). Despite a decade of intensive studies, much about the PM health effects problem, especially the cardiovascular effect, is still not well understood.

The present study is designed to test the hypothesis that diesel exhaust exposure alters the outcome of adverse cardiac events, specifically on the cardiac autonomic function and systemic inflammation. Another goal is to evaluate the efficacy of omega-3 fatty acids as protection against the cardiovascular effects of diesel exhaust exposure. Omega-3 polyunsaturated fatty acids have several potentially cardioprotective effects, including antiarrhythmic, antithrombotic, antiatheroscleotic, anti-inflammatory, improving endothelial function, lowering blood pressure, and lowering plasma triglyceride concentrations (see review in (18)). Decreased HRV has been used to predict an increased risk of cardiovascular morbidity and mortality and several studies suggest that fish oils may improve the autonomic function, including HRV and baroreflex sensitivity. Fish oil supplementation was shown to reduce the incidence of sudden cardiac death after myocardial infarction in humans due to its anti-arrhythmic effect (19-21). Fish oil

supplementation (2 g/d) has been found to significantly increase HRV compared with 2 g/d of soy oil control in elderly subjects (22). A clinical trail with 2 g/d of fish oil supplementation has been shown to prevent HRV decline related to PM exposure in an elderly population (23). No controlled human studies have been conducted to investigate the potential protective effect of fish oils on the adverse health effects of exposure to air pollutants.

The disturbance of the autonomic control of the heart is a documented effect of air pollutants on the cardiovascular system. The disturbances are proposed to be mediated by air pollutant-induced oxidative stress. HRV was reduced in subjects with GSTM1 null compared to GSTM1 positive genotype (24). Another enzyme called GSTP1 is also associated with anti-oxidants effects and we will determine the optimal concentration of diesel exhaust in the pilot study in the most vulnerable subgroup with GDTM1 null and 3 GSTP1 codon 105 genotypes (Ile/Ile, Ile/Val, and Val/Val). Therefore, the role of GSTs in mediating the cardiovascular effects following acute diesel exhaust exposure will also be studied in the main protocol in GSTM1 null and GSTM1 positive healthy older adults.

U.S. EPA regulations concerning diesel emissions, mandated by the Clean Air Act, have resulted in dramatic decreases in emissions from diesel engines since 1988, with the last set of significant engine modifications occurring in 1995 (EPA, 1998). The respiratory effects of diesel exhaust have been characterized in humans but the cardiovascular effects have not been well examined. An experimental assessment of the health effects on cardiovascular system to diesel exhaust in humans and identification of potential prevention strategies are high priorities for US EPA regulatory offices. In summary, the study described in this protocol will examine the health effects of exposure to diesel exhaust in healthy adult human subjects and will evaluate the value of fish oil supplementation as a mitigation strategy.

1. Sydbom, A., Blomberg, A., Parnia, S., Stenfors, N., Sandstrom, T. and Dahlen, S.E. (2001). Health effects of diesel exhaust emissions. Eur Respir J 17:733-746.
2. Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L. and Samet, J.M. (2006). Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295:1127-1134.
3. Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr. and Tager, I. (2004). Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655-2671.
4. Hoek, G., Brunekreef, B., Fischer, P. and van Wijnen, J. (2001). The association between air pollution and heart failure, arrhythmia, embolism, thrombosis, and other cardiovascular causes of death in a time series study. Epidemiology 12:355-357.
5. Peters, A., Dockery, D.W., Muller, J.E. and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. Circulation 103:2810-2815.
6. Johnson, R.L., Jr. (2004). Relative effects of air pollution on lungs and heart. Circulation 109:5-7.
7. Pope, C.A., 3rd, Verrier, R.L., Lovett, E.G., Larson, A.C., Raizenne, M.E., Kanner, R.E., Schwartz, J., Villegas, G.M., Gold, D.R. and Dockery, D.W. (1999). Heart rate variability associated with particulate air pollution. Am Heart J 138:890-899.
8. Peters, A., Doring, A., Wichmann, H.E. and Koenig, W. (1997). Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582-1587.
9. Brook, R.D., Brook, J.R., Urch, B., Vincent, R., Rajagopalan, S. and Silverman, F. (2002). Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. Circulation 105:1534-1536.
10. Peters, A., Frohlich, M., Doring, A., Immervoll, T., Wichmann, H.E., Hutchinson, W.L., Pepys, M.B. and Koenig, W. (2001). Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. Eur Heart J 22:1198-1204.
11. Schwartz, J. (2001). Air pollution and blood markers of cardiovascular risk. Environ Health Perspect 109 Suppl 3:405-409.

12. Sandhu, R.S., Petroni, D.H. and George, W.J. (2005). Ambient particulate matter, C-reactive protein, and coronary artery disease. Inhal Toxicol 17:409-413.
13. Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., Brook, R.D., Aguinaldo, J.G., Fayad, Z.A., Fuster, V., Lippmann, M., Chen, L.C. and Rajagopalan, S. (2005). Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA 294:3003-3010.
14. Le Tertre, A., Medina, S., Samoli, E., Forsberg, B., Michelozzi, P., Boumghar, A., Vonk, J.M., Bellini, A., Atkinson, R., Ayres, J.G., Sunyer, J., Schwartz, J. and Katsouyanni, K. (2002). Short-term effects of particulate air pollution on cardiovascular diseases in eight European cities. J Epidemiol Community Health 56:773-779.
15. Salvi, S., Blomberg, A., Rudell, B., Kelly, F., Sandstrom, T., Holgate, S.T. and Frew, A. (1999). Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. American journal of respiratory and critical care medicine 159:702-709.
16. Behndig, A.F., Mudway, I.S., Brown, J.L., Stenfors, N., Helleday, R., Duggan, S.T., Wilson, S.J., Boman, C., Cassee, F.R., Frew, A.J., Kelly, F.J., Sandstrom, T. and Blomberg, A. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27:359-365.
17. Mills, N.L., Tornqvist, H., Robinson, S.D., Gonzalez, M., Darnley, K., MacNee, W., Boon, N.A., Donaldson, K., Blomberg, A., Sandstrom, T. and Newby, D.E. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930-3936.
18. Din, J.N., Newby, D.E. and Flapan, A.D. (2004). Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 328:30-35.
19. Leaf, A., Kang, J.X., Xiao, Y.F. and Billman, G.E. (2003). Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 107:2646-2652.
20. Leaf, A., Albert, C.M., Josephson, M., Steinhaus, D., Kluger, J., Kang, J.X., Cox, B., Zhang, H. and Schoenfeld, D. (2005). Prevention of fatal arrhythmias in high-risk subjects by fish oil n-3 fatty acid intake. Circulation 112:2762-2768.
21. Marchioli, R., Barzi, F., Bomba, E., Chieffo, C., Di Gregorio, D., Di Mascio, R., Franzosi, M.G., Geraci, E., Levantesi, G., Maggioni, A.P., Mantini, L., Marfisi, R.M., Mastrogiuseppe, G., Mininni, N., Nicolosi, G.L., Santini, M., Schweiger, C., Tavazzi, L., Tognoni, G., Tucci, C. and Valagussa, F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. Circulation 105:1897-1903.
22. Holguin, F., Tellez-Rojo, M.M., Lazo, M., Mannino, D., Schwartz, J., Hernandez, M. and Romieu, I. (2005). Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 127:1102-1107.
23. Romieu, I., Tellez-Rojo, M.M., Lazo, M., Manzano-Patino, A., Cortez-Lugo, M., Julien, P., Belanger, M.C., Hernandez-Avila, M. and Holguin, F. (2005). Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter. American journal of respiratory and critical care medicine 172:1534-1540.
24. Schwartz, J., Park, S.K., O'Neill, M.S., Vokonas, P.S., Sparrow, D., Weiss, S. and Kelsey, K. (2005). Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. American journal of respiratory and critical care medicine 172:1529-1533.
25. Lauer, M.S., Pothier, C.E., Chernyak, Y.B., Brunken, R., Lieber, M., Apperson-Hansen, C. and Starobin, J.M. (2006). Exercise-induced QT/R-R-interval hysteresis as a predictor of myocardial ischemia. Journal of electrocardiology 39:315-323.

A.4.3. **Subjects.** *You should describe the subject population even if your study does not involve direct interaction (e.g., existing records).* Specify number, gender, ethnicity, race, and age. Specify whether subjects are healthy volunteers or patients. If patients, specify any relevant disease or condition and indicate how potential subjects will be identified.

Subjects for this study will be healthy 50-75 year-old male and female subjects. Our recruitment goal includes approximately 6 subjects with both GSTM1 null and GSTP1 codon 105 (Ile/Ile, Ile/Val, and Val/Val; n=2 each) genotypes for the pilot study and 60 subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) for the main study. Subjects will be recruited through the Westat Corporation (see section B1 below).

A.4.4. **Inclusion/exclusion criteria.** List required characteristics of potential subjects, and those that preclude enrollment or involvement of subjects or their data. Justify exclusion of any group, especially by criteria based on gender, ethnicity, race, or age. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided.

Inclusion criteria:

- Age 50-75 years old generally healthy male and female.
- Normal resting ECG.
- Oxygen saturation greater than 94% at the time of physical exam.

Exclusion criteria (pilot study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin. (delete)
- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors. (delete)
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Have an allergy to latex.

Exclusion criteria (main study):

- A history of angina, cardiac arrhythmias, and ischemic myocardial infarction or coronary bypass surgery.
- Cardiac pacemaker.
- Uncontrolled hypertension (> 150 systolic, > 90 diastolic).
- Neurodegenerative diseases such as Parkinson's and Alzheimer disease.
- A history of chronic illnesses such as diabetes, cancer, rheumatologic diseases, immunodeficiency state, known cardiovascular disease, chronic respiratory diseases such as chronic obstructive pulmonary disease or severe asthma.
- History of bleeding diathesis.
- Currently taking HMG-CoA reductase inhibitors for hyperlipidemia including lovastatin, pravastatin, simvastatin, and atorvastatin. (delete)

- Currently taking β-blockers to control hypertension and/or arrhythmias.
- Currently taking ACE inhibitors.(delete)
- Use of oral anticoagulants.
- Participants must refrain from all over-the-counter NSAIDs for a period of two weeks prior to exposure. Low-dose aspirin will be acceptable. Medications not specifically mentioned here may be reviewed by the investigators prior to a participant's inclusion in the study.
- Allergies to fish or omega-3 fatty acids.
- Subjects are on prescriptions taking omega-3 fish oil as therapy.
- Subjects will be required to avoid taking omega-3 fatty acids or having more than one 4-6 oz/serving of all types of fish and shellfish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for two weeks before and during the study.
- Subjects will be required to avoid taking antioxidants (e.g., beta-carotene, selenium, vitamin C, vitamin E, zinc) for two weeks before and during the study.
- Subjects will be required to use olive oil exclusively for cooking, dressings, and sauces during the study and to avoid other vegetable oils because of their omega-3 contents.
- Female subjects currently taking estrogen replacement therapy.
- Because of reported cardioprotective effects of red wine, subjects will be prohibited from drinking red wine during the study.
- Subjects who are currently smoking or have smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year).
- No exposure will be conducted within 4 weeks of a respiratory tract infection.
- Subject is pregnant, attempting to become pregnant or breastfeeding.
- Have an allergy to latex.

Use of other medications will be evaluated on a case-by-case basis. There is the potential that an individual's current medication use will preclude them from participating in the study at the current time, but they may be reassessed and potentially rescheduled for participation at a later time.

A.4.5. **Full description of the study design, methods and procedures.** Describe the research study. Discuss the study design; study procedures; sequential description of what subjects will be asked to do; assignment of subjects to various arms of the study if applicable; doses; frequency and route of administration of medication and other medical treatment if applicable; how data are to be collected (questionnaire, interview, focus group or specific procedure such as physical examination, venipuncture, etc.). Include information on who will collect data, who will conduct procedures or measurements. Indicate the number and duration of contacts with each subject; outcome measurements; and follow-up procedures. If the study involves medical treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls.

Pilot study: Approximately 6 subjects will undergo 3 exposures to air containing approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts sequentially for 2 hours in an exposure chamber. There will be a 2-week interval between exposures. We anticipate that there will be 6 subjects at each concentration of diesel exhaust exposure during the course of this pilot study. However, circumstances beyond our control may arise (i.e. subject/subjects might drop off due to illness and/or other reasons during the study) which may prevent finishing the proposed 3

exposures, and then we will have to add new subject/subjects at that point. Each new subject will have 3 exposures at approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ diesel exhausts. Subjects who withdraw from the study will be compensated for all procedures and time completed to that point (See the pilot study design flow chart).

The purpose of the pilot study is to identify safe and measurable cardiovascular effects of diesel concentration. Subjects will have the second exposure in about two weeks after the first exposure. All six subjects will complete the 200 $\mu g/m^3$ exposure before the 300 $\mu g/m^3$ exposures are started. Our primary endpoints in the pilot study include HRV, pulmonary function, and biomarkers such as neutrophils, platelets, fibrinogen, c-reactive protein, lactate, ferretin, plasminogen activator inhibitor, and α1-antitrypsin. We will also assess the endothelial cell function by brachial artery ultrasound and finger artery Endo-Pat. We will analyze these endpoints after finishing the 200 $\mu g/m^3$ diesel exposures and determine whether we will continue the 300 $\mu g/m^3$ concentration exposure. For example, if we find effects at approximately 200 $\mu g/m^3$ of diesel concentration then we will use that concentration for our main study. We may not need to test subjects at the next higher proposed concentrations. We will carefully monitor the symptoms that subjects may develop during the exposure and over the following 24 hour period. The symptoms include chest pain, dyspnea, pallor, ataxia, and significant cardiac arrhythmias. In addition, analyses of blood samples taken after exposure will be scrutinized for abnormalities, including elevated lactate level, changes in fibrinogen concentrations, as well as significant increases in inflammatory markers such as neutrophil counts and the concentration of c-reactive protein. Symptoms and/or changes detected in the blood samples that are considered clinically significant could prompt the study physicians to stop the study. Therefore, we will select the diesel concentration based solely on safety considerations.

Main study: This will be a randomized double-blinded study. Sixty study subjects (30 subjects GSTM1 positive and 30 subjects GSTM1 null) will be given, in a double-blind fashion, a container filled with enteric-coated either 2g/day of fish oil or 2g/day of olive oil capsules for 15 days. Fish oil and olive oil supplements will be provided by Pharmavite, LLC. Each 1000 mg of these fish oil capsule contains at least 65% of omega-3 fatty acid with 25-35% of eicosapentaenoic acid (EPA) and 30-65% of docosahexaenoic acid (DHA). Each 1000 mg of these olive oil capsule contains less than 1% of omega-3 fatty acid with 73% of oleic acid and 12% of palmitic acid. There will be four groups of subjects in this study with 15 subjects in each group: the first group is fish oil supplement in GSTM1 positive subjects, the second group is fish oil supplement in GSTM1 null subjects, the third group is olive oil supplement in GSTM1 positive subjects, and the fourth group is olive oil supplement in GSTM1 null subjects. In the exposure study, each subject will be exposed to air containing approximately optimum diesel exhaust for 2 hours in an exposure chamber. (See the main study design flow chart).

Subject Qualification

Screening: Subjects will be recruited by the Westat Corporation (see section B1 below). During an initial telephone interview, the subjects will receive information regarding the study and their eligibility status will be assessed. Subjects whose responses indicate that they are likely to meet the criteria will be scheduled for a genetic screening appointment in the medical station in the USEPA Human Studies Facility (HSF). At that time a genotype screening informed consent form and a consent form for storing blood with identifying information will also be signed. The subjects identified from a previous study (see page 26, methods of recruiting) will not need genotype screening. Subjects will then undergo a brief medical history screening and blood

pressure measurement. Subjects will then have approximately 50 ml of blood collected in the pilot study and approximately 80 ml of blood collected in the main study. A portion of the blood will be used for genetic screening of GSTM1 and/or GSTP1 Ile105 Val polymorphism and another portion of the blood will be used for the cholesterol levels, fatty acid analysis, and biochemistry analysis. Subjects will be given a copy of the Medical History Form to be filled out and mailed back after they are selected on the genotype.

- ***GSTM1/GSTP1 genotype screening***: A portion of the blood sample (about 5 ml) will be used for genotyping of GSTM1 and GSTP1. Total RNA will be isolated from the blood sample and polymeric chain reaction (PCR) will be run to determine if the subject carries a GSTM1 positive or null gene and GSTP1 Ile105Val polymorphism. Since the pilot study only seeks people that are GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotypes, and since the joint GSTM1 null and GSTP1 (Ile/Ile, Ile/Val, and Val/Val) genotype is present in about 8%, 17%, and 14% respectively of the population, it is likely that about 80-90 % of the subjects will be excluded in this pilot study but they will be informed to participate in our future study which will enroll both GSTM1 positive and null genotypes. Those subjects with GSTM1 positive will not be asked to continue with the study and will be compensated for their time. Since the main study seeks to enroll equal numbers of people that are GSTM1 positive and null, it is likely that as the study nears completion, we will still be seeking people with the null allele but not with the sufficient allele. If that is the case, those subjects will not be asked to continue with the study and will be compensated for their time.

- ***Food frequency questionnaire in the main study***: Subjects will be given a dietary and medication instruction sheet to follow. Subjects will also take a Food Frequency Questionnaire (FFQ) to assess usual intake of omega-3-rich food sources. Completed FFQs will be sent to Dr. Susan Steck at the university of south carolina for analyses. Subjects will be asked to eliminate use of nutritional supplements containing omega-3 fatty acids and avoid all type of fish and shell fish, walnuts, flaxseeds and flaxseed oil, rapeseed oil, canola oil, soybeans and soy products, omega-3 fortified eggs, and cod liver oil for **two weeks** prior to their enrollment in this study if they are qualified.

Physical exam: Subjects who are not excluded during the genetic screening will be scheduled for a physical examination in the HSF. Those subjects who are genetically screened but are not eligible for the pilot study, may be included in the main study if they are interested in participating. During this visit, subjects will sign an informed consent for a physical and the medical history form completed that contains information on general personal and family medical history. Subjects will then undergo an abbreviated physical exam, blood pressure, pulse oximetry, and 12-lead electrocardiogram (ECG) to screen for baseline cardiac arrhythmias and ST segment. A menstrual history will be collected on all female subjects.

Training session: Those subjects who are not excluded on the basis of the physical exam and genetic screening will undergo a training session to familiarize them with the study protocol. At that time the study protocol will be outlined and informed consent obtained to initiate the study. The subjects will ask any questions they might have regarding their participation in the study. Their first exposure session will be scheduled and fish oil or olive oil supplements will be administrated. Subjects will then undergo spirometry for pulmonary function testing. Pregnancy tests will be administrated to any female subjects who may have child-bearing potential on the

training day and on the exposure day if more than 7 days since last pregnancy test it will be repeated.

- ***Fish oil or olive oil pretreatment and dietary assessment in the main study:*** Subjects will be given an approximately 15 days supply of either fish oil or olive oil supplements randomly assigned. Subjects will be asked to take fish oil or olive oil supplements at dinner. Subjects will undergo a dietary assessment and dietary counseling. The dietary assessment is to (1) assess compliance with the dietary restrictions, and (2) measure intake of energy and nutrients that may confound the relationship between omega-3 and HRV in order to potentially control for these during the statistical analyses phase. Subjects will be asked to keep two 3-day food records (one 3-day record per week in the study). The 3-day food records should include three consecutive days of intake, with two days being weekdays (Monday through Friday) and one day being a weekend day (Saturday or Sunday). Subjects will be asked to record everything consumed during the 3-day period, including all foods, beverages and nutritional supplements. We will provide a handout for the subjects with instruction on how to complete the record, including instruction on how to estimate portion sizes with photographs of various portion sizes, at the beginning of the study. Subjects should be aware that they may be contacted by telephone for clarification of ambiguous information. Completed records will be sent to Dr. Susan Steck at USC immediately upon completion for timely review and for nutrient composition analyses using the NDSR (University of Minnesota Nutrition Data System for Research) software.

Subject will be rescheduled if they have experienced an illness and they can not continue taking the supplements during the 15 day of supplementation period. We will give the subjects another 15 days supply of supplements and they will need to restart the supplements when their healths are back to normal.

We anticipate performing several clinical procedures during the course of this study that include primary, secondary and exploratory endpoints. However, circumstances beyond our control may arise (i.e. equipment failure) which may prevent performing a specific procedure on an individual subject. It is possible that not all procedures will be performed on every subject. If we are unable to perform a procedure which is a primary endpoint, then the patient will be compensated for all procedures and time completed on that day and rescheduled. If, however, a procedure involving collecting data in support of a secondary or exploratory endpoint could not be performed and this procedure is also a source of compensation for the subject, the subject would be compensated for that procedure but not rescheduled to make up the procedure.

- ***Baseline HRV measurement*:** In the pilot and main study, subjects will have their baseline 24-hour heart rate viability (HRV) measured by Holter monitor on the training day. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Subjects will be released while wearing the Holter monitor and return to the HSF the next day to get the Holter monitor removed.

Exposure Day

In order to participate in this study, subjects will be required to:

- Avoid smoke and fumes for 24 hours before all visits.
- Avoid drinking alcohol 24 hours before all visits.
- Avoid strenuous exercise for 24 hours prior to and after all visits.
- Not to eat pan fried and/or grilled foods after midnight prior to the exposure day.
- Not to consume caffeine for 12 hours prior to all study visits.
- Eat a light breakfast on the exposure day.

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (blood pressure, HRV, endothelial cell function by brachial artery ultrasound (BAU) and possibly by Endo-PAT, pulmonary function by spirometry, and blood sampling) will be completed.

- ***HRV measurement in both pilot and main studies*** will be done by a Holter monitor. Electrodes for HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. These leads will be connected to a Holter monitor and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed, and removed when the patient leaves for the day. The subject will then be allowed to relax for 20 minutes in a reclined

- ***Brachial artery ultrasound in both the pilot and main studies:*** Brachial artery ultrasound (BAU) to evaluate flow-mediated dilatation will be performed in the North Carolina Memorial Hospital (NCMH) General Clinical Research Center (GCRC) using a 12.5 MHz imaging probe interfaced with an ATL HDI 5000 ultrasound machine. The diameter of the brachial artery will be measured at baseline, during reactive hyperemia and after administration of sublingual nitroglycerin. The subject will lie supine, and a pneumatic tourniquet will be placed around the right upper arm proximal to the target artery. Gated baseline images of the brachial artery will be acquired after 15 minutes of supine rest. The pneumatic cuff will then be inflated to a pressure of 200 mm Hg for 5 minutes, and increased flow will be induced by sudden cuff deflation. A second scan will be performed following deflation. The subject will rest another 10 minutes and a third ultrasound scan will be performed. Sublingual nitroglycerin (~0.4 mg) will be administered, followed in three to four minutes by the final ultrasound study. Subjects will then rest quietly for 5 minutes. Images of the brachial artery will be acquired and stored on a personal computer, and subsequently analyzed using a semi-automated offline quantification system.

- ***Peripheral endothelial function by Endo-PAT in both pilot and main studies***: Another non-invasive index finger arterial endothelial function might be measured by Endo-PAT 2000 (Endo-PAT) (Itamar Medical Ltd, Caesarea, Israel) at HSF and we will compare the findings with BAU. If we find that the pattern of endothelial function measured by PAT is similar to that of BAU in the middle of the study, then we will drop the BAU measurements. PAT is a technology that captures a beat-to-beat plethysmographic recording of the finger arterial pulse wave amplitude (PWA) with pneumatic probes. The PAT probes will be applied to the index fingers of both arms in the subject. The pre-occlusion baseline finger arterial PWA recordings will be acquired for 5 minutes at rest. A reactive hyperemia procedure will be performed by occluding the brachial artery of one

arm by inflating a pressure of 200 mm Hg for 5 minutes and increased flow will be induced by sudden cuff deflation. The PWA recordings will be measured continuously during suprasystolic occlusion and deflation reactive hyperemia. PAT measurement will be displayed, stored, and analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel).

- ***Pulmonary function in both pilot and main studies*** will be measured by spirometry.
- ***Blood sampling in both pilot and main studies*:** approximately 50 ml of blood samples will then be collected in the pilot study and approximately 80 ml of blood samples will then be collected in the main study.
- ***Urine sampling in both the pilot and main studies:*** approximately 30 ml of urine samples will be collected.
- ***Symptoms questionnaire*** before exposure will be collected.

In the pilot study: Subjects will then enter the diesel exposure chamber for 3 sequentially exposures at concentrations approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$ for 2 hours each with a about 2-week interval between exposures. In the main study: Subjects will have only one exposure at an optimum concentration of diesel exhaust that will be identified from the pilot study.

Exposure: All exposures will be carried out at the EPA Human Studies Facility on the UNC campus. Subjects will be monitored continuously by the EPA personnel. A duty physician will be available. The subjects will be able to end their exposure and exit the chamber at any time if they choose to end their participation in the study. Total exposure time will be 2 hours. The exposure atmosphere will be at approximately 40 $\pm$ 10% RH and approximately 22 $\pm$ 2 °C. The diesel exhaust will be generated from a 6-cylinder, 205 hp, 5.9 L displacement diesel engine (Cummins, Columbus IN) mounted on a vehicle located outside the Human Studies Facility, and subsequently introduced into the exposure chamber after different dilutions with clean filtered and humidified air by approximately 1/30th to give a chamber concentration of approximately 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m^3$. The monitoring analyzer registers a concentration greater than 100 ug/m^3 above the target concentration as the exposure limit. Specifically, exposures will be terminated at values $\geq$200, 300 or 400 during runs in which the exposure targets are 100, 200 and 300 ug/m^3, respectively. The running sum of the 2-minute average concentrations displayed by the monitoring analyzer during the 2 hr exposure period exceeds 18,000 ug/m^3. This safety limit is specifically designed to address exposures for which the target concentration is 300 ug/m^3. It will prevent an inhalational exposure greater than 600 ug, which assumes that the resting subject will inhale 2 m^3 during the 120 min exposure. It is expected that 75 to 95% of the diesel exhaust PM from this type of engine will be centered around approximately 0.1-0.2 μm. Preliminary testing has shown the mean particle size to be approximately 0.14 μm. Levels of carbon monoxide, and oxides of nitrogen (NOx; mainly NO, and some NO2), will be kept under 10 ppm CO, 15 ppm NO, and 2.5 ppm NO_2 or the exposure will be terminated. [The OSHA 8 hr time-weighted average (TWA) for these substances are: carbon monoxide=50 ppm; NO2=5 ppm; NO=25 ppm; The American Conference of Governmental Industrial Hygienists (ACGIH) 8 hr threshold limit values (TLVs) are: NO=25 ppm, NO2=3 ppm, and CO=25 ppm]. Diesel fuel used for the study will be purchased as a certified fuel (Chevron Phillips Chemical Co., Borger TX, 0.05 LS Certification Fuel, type II). This certified fuel represents diesel fuel composition that is typically available at gas stations for most regions of the country except for California. Subjects will enter the exposure chamber (about 6 ft x 6 ft x 8 ft). Particle mass will be measured with a tapered element oscillating microbalance (TEOM) and/or a Data-RAM, and chamber PM concentrations

during exposure further validated by weighing filters obtained from versatile air pollution samplers (VAPS) impacters. Particle size distribution will be determined by scanning mobility particle size (SMPS) not during the subject exposure but at regular intervals (e.g., monthly) to show that the size distribution does not change radically. Filter samples will also be analyzed for chemical composition of particles. The minute ventilation during exposure will be measured. Blood pressure, heart rate, and oxygen saturation will be measured during the exposure period.

Post-exposure: Subjects will be released while wearing the Holter monitor. Symptoms questionnaire after exposure will be collected. Blood pressure, lung function, endothelial cell function, and heart rate variability will be measured and approximately 50 ml of blood and 30 ml of urine samples will be collected in the pilot study and approximately 80 ml of blood will be collected in the main study. Approximately 30 ml of urine samples will be collected in both the pilot and main studies.

Eighteen Hours Post-exposure: Eighteen hours after the exposure, the subjects will return to the HSF to undergo a brief medical evaluation, including blood pressure, spirometry (pulmonary function testing) and endothelial cell function measurements. The subject will then be allowed to relax for 20 minutes in a reclined position, after which a 10-minute resting HRV measurement will be obtained, and approximately 50 ml of blood will be collected in the pilot study and approximately 80 ml of blood will be taken in the main. Holter monitor will be removed.

OUTCOMES:

Pulmonary Function will be measured before and after exposure. Subjects who have recent abdominal and/or eye surgery, and with any types of hernia should not be asked to have pulmonary function tested. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard procedure published by the American Thoracic Society. In addition, regional DLCO and pulmonary capillary blood flow (Qc) will be obtained by the intrabreath technique using the same system.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. premature atrial complex, premature ventricular contractions, bradycardia, and tachycardia).

Flow-Mediated Dilatation (brachial artery ultrasound and finger arteries Endo-PAT). Changes in diameter of arteries caused by reactive hyperemia (endothelium-dependent vasodilatation) and administration of sublingual nitroglycerin (endothelium-independent vasodilatation) will be expressed as a percent change in diameter relative to resting baseline values.

Peripheral Venous Blood Sample. Before, immediately after (within 1 hr after exposure ends), and approximately 18 hrs after exposure, blood (about 50 ml in the pilot study about 80 ml in the main study) will be drawn by standard venipuncture technique. For venipuncture, the site is prepared with isopropyl alcohol. A tourniquet is applied. Blood is drawn from an antecubital or other appropriate vein. Endpoint measurements will include, but not be limited to, the following:

omega-3 fatty acid level, biomarkers for specific and non-specific immune responses, coagulation factors, vasoactive factors, and soluble components of PM (e.g. transition metals).

In the main study, our primary endpoints will be HRV measurement and peripheral venous blood sampling. Our secondary endpoints will be endothelial cell function and pulmonary function measurements.

A.4.6. **Benefits to subjects and/or society.** Describe any potential for direct benefit to individual subjects, as well as the benefit to society based on scientific knowledge to be gained; these should be clearly distinguished. Consider the nature, magnitude, and likelihood of any direct benefit to subjects. If there is no direct benefit to the individual subject, say so here and in the consent form (if there is a consent form). Do not list monetary payment or other compensation as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination, including blood work, brachial artery ultrasound, spirometry, and an ECG. Subjects will have full access to these records. They will also gain knowledge about their responsiveness to a diesel exhaust and the supplement of fish oil might protect them from air pollutant.

For society, this study will provide new information on the effects of diesel exhaust on regional lung function, inflammation, and the cardiovascular system. Data from this study will help the US EPA better understand the components of air pollution that are responsible for increasing morbidity and mortality of cardiopulmonary fatality so that federal regulations can be properly set. Findings from this study will also become the potential to contribute to devising effective strategies aimed at protecting millions from the untoward effects of these pollutants.

A.4.7. **Full description of risks and measures to minimize risks.** Include risk of psychosocial harm (e.g., emotional distress, embarrassment, breach of confidentiality), economic harm (e.g., loss of employment or insurability, loss of professional standing or reputation, loss of standing within the community) and legal jeopardy (e.g., disclosure of illegal activity or negligence), as well as known side effects of study medication, if applicable, and risk of pain and physical injury. Describe what will be done to minimize these risks. Describe procedures for follow-up, when necessary, such as when subjects are found to be in need of medical or psychological referral. If there is no direct interaction with subjects, and risk is limited to breach of confidentiality (e.g., for existing data), state this.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is present whenever a subject is undergoing any procedure. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. HSF has a fully stocked medical station and the University of North Carolina Hospital is a short distance from the HSF. On subsequent days after exposure subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single

inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.

- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Brachial artery ultrasound and finger arteries Endo-PAT:** There are no known risks associated with imaging of the brachial artery and index finger arteries. However, intermittent brief occlusion of blood flow to the forearm may cause mild discomfort and temporary sensations such as tingling and numbness until the blood pressure cuff is released. Approximately 0.5 % of participants develop painless petechiae in the arm which is examined and these resolve within a few days. Sublingual nitroglycerin for the brachia artery ultrasound measurement is a potent vasodilator, and may be associated with headache, flushing, and transient hypotension. These results are short-lived because the peak plasma concentration occurs within 4 minutes of administration and the plasma half-life is approximately 5 minutes. To minimize the risk of hypotension, the subject will remain lying down for 10 minutes after receiving nitroglycerin. In addition, individuals who may be at risk of excessive blood pressure lowering (i.e. individuals who have baseline systolic blood pressure < 90 mm Hg, or who have obstruction of the left ventricular outflow tract due to aortic stenosis or a dynamic outflow gradient) will be excluded. Allergic reactions to nitroglycerin have been reported, but are rare.
- **Venipuncture** will be done by insertion of the needle and may cause minor discomfort at the site of injection and there is a possibility that a bruise will form which may be painful for 2-3 days. It is possible that the subject may feel lightheaded or even faint due to anxiety about the blood draw. Rarely, a skin infection may occur. To minimize these risks, blood is drawn by trained medical professionals. Subjects are in a reclined position and closely monitored for any signs of faintness, given liquids and food to eat if requested, and only allowed to leave the facility after a 15-minute waiting period to make sure they are stable.
- **Fish oil/olive oil supplementation** Dietary supplements with fish oil/olive oil are relatively safe as a whole. We have excluded subjects who have fish allergy. Allergic reactions to olive oil have been reported, but are rare. High-dose fish oil may increase in LDL, bleeding times, and worsening of glycemic control in diabetics (who will be excluded from the study). Therefore, 2g/day in this study should not impose a significant risk to subjects who are eligible to participate. Fish oil is known to cause gastrointestinal upset in some people. We will use enteric-coated fish oil tablets to mitigate this side effect of fish oil supplementation.
- **Diesel exposure** The amount of diesel exhaust used for exposure in this study would be equivalent to concentrations of diesel exhaust particulate matter that would be encountered at busy intersections in large urban areas. Diesel particulate concentrations proposed for the pilot study will be 100 $\mu g/m^3$, 200 $\mu g/m^3$, and 300 $\mu g/m3$ and the optimum concentration will be used for the main study. These concentrations are the occupational levels for some truck drivers (generally about 100-300 $\mu g/m^3$) and 1-2 mg/m^3 for some mines. Some areas of heavily trafficked streets in Los Angeles and New York City have had diesel exhaust levels >20 $\mu g/m^3$, and nearby residents could have exposure to these concentrations over several hours. Controlled diesel exhaust exposure studies in Sweden using 300 $\mu g/m^3$ DEP have shown lung inflammatory effects not unlike effects observed with numerous ozone exposure studies performed here at the EPA facility on the UNC campus during the past 20 yr. Diesel exhaust particles contain some probable carcinogenic polycyclic aromatic hydrocarbons, which in high enough concentrations and/or with repeated exposures may induce tumors. Diesel exhaust also contains aldehydes, some of which are possibly carcinogenic in high enough dose and with long enough exposure. However, the

exposure concentrations to be used in this protocol are minimal. Overall, it appears that at the low DEP concentration to be given one time for the exposure in this study, the risk of cancer, if it exists at all, is extremely low and certainly no more than what one would experience if one were to visit for a few days a particulate-polluted city in the US such as Los Angeles or New York City.

Health effects of acute exposures to air pollution particles are not well defined but possibly include chest pain, mild dyspnea, headache, cough, and, in some subjects, wheezing. All of these effects resolve spontaneously within hours of exposure cessation. During exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have EKG leads attached during the exposure to monitor cardiac function during exercise. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for their participation up to that point. The investigators or duty physician will end the exposure if the subject is found to be suffering from any significant adverse effect. The small pilot study will allow us to monitor the adverse effects of diesel exhaust closely and we will determine whether we will go to the next higher concentration based on the analysis. We will be able to identify a safe concentration of diesel exhaust that induces a measurable change in our study endpoints. No major adverse health effects were observed in a recently completed study that exposed subjects (18-35 yr old, healthy volunteers) to concentrated Chapel Hill air particles here at the EPA facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; UNC IRB# 95-EPA-310). The maximum PM concentration reached 311 $\mu g/m^3$ during the exposure. The data from this exposure study suggest that normal healthy subjects tolerate inhalation of concentrated ambient PM fairly well, and support the idea that exposures to lower PM concentrations, including DEP, will be fairly safe in terms of cardiopulmonary responses. For safety reasons, pulse oximetry will be performed during the exposures to diesel exhaust and filtered air, and the subject withdrawn from the chamber if the O_2 saturation value is <89%. Additionally subjects will have carboxyhemoglobin values determined within 1 hr after an exposure and kept at the Medical Station if levels are >20% (where performance of everyday cognitive tasks may be jeopardized) until levels become <20%; but because the CO levels are expected to be 2-4 ppm [based on preliminary chamber characterizations], levels are expected to be < 5%.

A duty physician is always onsite to respond to an emergency. Fully equipped resuscitation equipment is available for use in the event of a cardiac or pulmonary emergency. Physicians at the University of North Carolina (UNC) Hospitals Emergency Room are also available to assist in treatment of an emergency.

Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

- **Confidentiality** Risk of breach of confidentiality is minimal. All subjects will be assigned a study number which will be used for data recording – not the subject's name. The study number is all that will be entered into computer databases. All paper files that may contain the subject's name or screening number are secure in the EPA building that has limited access 24 hours/day. Any abnormal medical findings (CBC, ECG, brachial artery ultrasound image, spirometry) will be discussed with the volunteer and the volunteer will be counseled to seek treatment from his/her

personal physician if indicated. Samples will be stored at the U.S. EPA HSF. A numeric coding system will be used to ensure that subjects cannot be directly identified from the samples alone.

A.4.8. **Data analysis.** Tell how the qualitative and/or quantitative data will be analyzed. Explain how the sample size is sufficient to achieve the study aims. This might include a formal power calculation or explanation of why a small sample is sufficient (e.g., qualitative research, pilot studies).

To test our hypothesis that diesel exhaust causes adverse cardiovascular effects and omega-3 fatty acid supplement pretreatment would attenuate the adverse cardiovascular effects. We also hypothesize that healthy older subjects with GSTM1 positive genotype have a lower cardiovascular risk than the subjects with GSTM1 null genotype when exposed to diesel exhaust. We will measure a number of endpoints (e.g., neutrophils, cytokine expression, and lung function). Several markers of the cardiovascular response to diesel will also be measured (e.g., blood pressure, HRV, brachial arterial blood flow rate, change in blood coagulation factors, platelets, fibrinogen, c-reactive protein, lactate, plasminogen activator inhibitor, ferretin, and α1-antitrypsin). In this study, our primary endpoints will be HRV measurement and peripheral venous blood markers. Our secondary endpoints will be endothelial cell function and pulmonary function measurements. Statistical data analyses will consist of ANOVA for continuous variables and rank sum tests for non- continuous variables to compare the effects of fish oil and olive oil, GSTM1 positive and null genotypes, and pre- and post-exposure. A p value of 0.05 or less will be considered significant.

The sample size was determined in order to detect a difference of 10% change in HRV assuming a standard deviation of 9%. Using GraphPad software, an N of 15 was derived using a power of 0.8 and an α of 0.05.

Other Exploratory Observations:
We hypothesize that GSTM1 null subjects will have increased responses to diesel exhaust compared to GSTM1 positive subjects. We estimate that GSTM1 null subjects will have a 4% decrease in brachial arterial blood flow rate, and GSTM1 positive subjects will have a 2% decrease. We also expect that GSTM1 positive subjects with fish oil supplements will have a 1% decrease in brachial arterial blood flow rate. We also estimate that GSTM1 null subjects will have 18% decrease in HRV, and GSTM1 positive subjects will have a 9% decrease. GSTM1 positive subjects with fish oil will have a 2% decrease in HRV.

Exploratory endpoints will include the effect of diesel exhaust on changes in: heart rate variability in both time and frequency domains, blood CBC and differential, fibrinogen and platelets, changes in IL 6 and IL 8 comparing pre-exposure with post-exposure values. Safety endpoints will include comparison of temperature, HRV, systolic and diastolic BP, respiratory rate, O_2 saturation and symptoms scores for pre- and post-exposure, and at 18 hours post-exposure.

A.4.9. **Will you collect or receive any of the following identifiers?** Does not apply to consent forms.

__ No __ Yes *If yes, check all that apply*:

a. _X_ Names
b. _X_ Telephone numbers
c. _X_ Any elements of dates (other than year) for dates directly related to an

individual, including birth date, admission date, discharge date, date of death. For ages over 89: all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 and older

d. _X_ Any geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code
e. __ Fax numbers
f. _X_ Electronic mail addresses
g. __ Social security numbers
h. _X_ Medical record numbers
i. __ Health plan beneficiary numbers
j. __ Account numbers
k. __ Certificate/license numbers
l. __ Vehicle identifiers and serial numbers (VIN), including license plate numbers
m. __ Device identifiers and serial numbers (e.g., implanted medical device)
n. __ Web universal resource locators (URLs)
o. __ Internet protocol (IP) address numbers
p. __ Biometric identifiers, including finger and voice prints
q. __ Full face photographic images and any comparable images
r. __ Any other unique identifying number, code, or characteristic, other than dummy identifiers that are not derived from actual identifiers and for which the re-identification key is maintained by the health care provider and not disclosed to the researcher

A.4.10. **Identifiers in research data**. Are the identifiers in A.4.9 above linked or maintained with the research data?

__ yes _X_ no

A.4.11. **Confidentiality of the data**. Describe procedures for maintaining confidentiality of the data you will collect or will receive. Describe how you will protect the data from access by those not authorized. How will data be transmitted among research personnel? Where relevant, discuss the potential for deductive disclosure (i.e., directly identifying subjects from a combination of indirect IDs).

No personal identifying information will be attached to the samples. No subjects will be identified in any report or publication about this study. Study samples will be stored in a secure room with restricted access. The sample will be prepared and stored indefinitely in a freezer for future testing. Portions of the sample may be shared with researchers at other scientific institutions or sent to outside clinical laboratories for analysis, however, only coded samples will be sent. All medical records generated during this study will be kept in the medical records office at the U.S. EPA Human Studies Facility.

A.4.12. **Data sharing.** With whom will *identifiable* (contains any of the 18 identifiers listed in question A.4.9 above) data be shared outside the immediate research team? For each, explain confidentiality measures. Include data use agreements, if any.

_X_ No one
__ Coordinating Center:
__ Statisticians:
__ Consultants:
__ Other researchers:
__ Registries:
__ Sponsors:
__ External labs for additional testing:
__ Journals:
__ Publicly available dataset:
__ Other:

A.4.13. **Data security for storage and transmission**. Please check all that apply.

For electronic data:

_X_ Secure network _X_ Password access __ Encryption
__ Other (describe):
__ Portable storage (e.g., laptop computer, flash drive)
Describe how data will be protected for any portable device:

For hardcopy data (including human biological specimens, CDs, tapes, etc.):

__ Data de-identified by research team (stripped of the 18 identifiers listed in question A.4.9 above)
__ Locked suite or office
_X_ Locked cabinet
__ Data coded by research team with a master list secured and kept separately
__ Other (describe):

A.4.14. **Post-study disposition of identifiable data or human biological materials**. Describe your plans for disposition of data or human biological specimens that are identifiable in any way (directly or via indirect codes) once the study has ended. Describe your plan to destroy identifiers, if you will do so.

Samples will be stored in a repository where only project members of the study will have access to the samples.

Part A.5. The Consent Process and Consent Documentation (including Waivers)

The standard consent process is for all subjects to sign a document containing all the elements of informed consent, as specified in the federal regulations. Some or all of the elements of consent, including signatures, may be altered or waived under certain circumstances.

- If you will obtain consent in any manner, complete **section A.5.1**.
- If you are obtaining consent, but requesting a waiver of the requirement for a signed consent document, complete **section A.5.2**.
- If you are requesting a waiver of any or all of the elements of consent, complete **section A.5.3**.
- If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* This is addressed in section B.2.

You may need to complete more than one section. For example, if you are conducting a phone survey with verbal consent, complete sections A.5.1, A.5.2, and possibly A.5.3.

A.5.1. **Describe the process of obtaining informed consent from subjects**. If children will be enrolled as subjects, describe the provisions for obtaining parental permission and assent of the child. If decisionally impaired adults are to be enrolled, describe the provision for obtaining surrogate consent from a legally authorized representative (LAR). If non-English speaking people will be enrolled, explain how consent in the native language will be obtained. Address both written translation of the consent and the availability of oral interpretation. *After you have completed this part A.5.1, if you are not requesting a waiver of any type, you are done with Part A.5.; proceed to Part B.*

The subject will be given an opportunity to read the consent. At that time a member of the study team (usually the PI) will verbally describe the study and the subject will have an opportunity to ask questions or address concerns about any aspect of the study. The subject will be given a copy of the signed consent form for his/her records.

A.5.2. **Justification for a waiver of *written* (i.e., signed) consent**. *The default is for subjects to sign a written document that contains all the elements of informed consent.* Under limited circumstances, the requirement for a signed consent form may be waived by the IRB if either of the following is true. *Chose only one:*

a. The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality (e.g., study topic is sensitive so that public knowledge of participation could be damaging). ___ yes ___ no

Explain.

b. The research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context (e.g., phone survey). ___ yes ___ no

Explain.

If you checked "yes" to either (and you are not requesting a waiver in section A.5.3) consent must be obtained orally, by delivering a fact sheet, through an online consent form, or be incorporated into the survey itself. Include a copy of the

consent script, fact sheet, online consent form, or incorporated document.

→ If you have justified a waiver of written (signed) consent (A.5.2), you should complete A.5.3 *only* if your consent process will not include all the other elements of consent.

A.5.3. **Justification for a full or partial waiver of consent.** *The default is for subjects to give informed consent.* A waiver might be requested for research involving only existing data or human biological specimens (see also Part C). More rarely, it might be requested when the research design requires withholding some study details at the outset (e.g., behavioral research involving deception). In limited circumstances, parental permission may be waived. This section should also be completed for a waiver of HIPAA authorization if research involves Protected Health Information (PHI) subject to HIPAA regulation, such as patient records.

__ Requesting **waiver of some elements** (specify; see SOP 28 on the IRB web site):
__ Requesting **waiver of consent entirely**
If you check either of the boxes above, answer items a-f.. To justify a full waiver of the requirement for informed consent, you must be able to answer "yes" (or "not applicable" for question c) to items a-f. **Insert brief explanations that support your answers.**

a. Will the research involve no greater than minimal risk to subjects or to their privacy? __ yes __ no
Explain.

b. Is it true that the waiver will *not* adversely affect the rights and welfare of subjects? *(Consider the right of privacy and possible risk of breach of confidentiality in light of the information you wish to gather.)* __ yes __ no
Explain.

c. When applicable to your study, do you have plans to provide subjects with pertinent information after their participation is over? *(e.g., Will you provide details withheld during consent, or tell subjects if you found information with direct clinical relevance? This may be an uncommon scenario.)* __ yes __ not applicable
Explain.

d. Would the research be impracticable without the waiver? *(If you checked "yes," explain how the requirement to obtain consent would make the research impracticable, e.g., are most of the subjects lost to follow-up or deceased?).* __ yes __ no
Explain.

e. Is the risk to privacy reasonable in relation to benefits to be gained or the importance of the knowledge to be gained? __ yes __ no
Explain.

If you are accessing patient records for this research, you must also be able to answer "yes" to item f to justify a waiver of HIPAA authorization from the subjects.

f. Would the research be impracticable if you could not record (or use) Protected Health Information (PHI)? *(If you checked "yes," explain how* not *recording or using PHI would make the research impracticable).* __ yes __ no
Explain.

Part B. Questions for Studies that Involve Direct Interaction with Human Subjects

→ ***If this does not apply to your study, do not submit this section.***

B.1. **Methods of recruiting.** Describe how and where subjects will be identified and recruited. Indicate who will do the recruiting, and tell how subjects will be contacted. Describe efforts to ensure equal access to participation among women and minorities. Describe how you will protect the privacy of potential subjects during recruitment. *For prospective subjects whose status (e.g., as patient or client), condition, or contact information is not publicly available (e.g., from a phone book or public web site), the initial contact should be made with legitimate knowledge of the subjects' circumstances. Ideally, the individual with such knowledge should seek prospective subjects' permission to release names to the PI for recruitment. Alternatively, the knowledgeable individual could provide information about the study, including contact information for the investigator, so that interested prospective subjects can contact the investigator.* Provide the IRB with a copy of any document or script that will be used to obtain the patients' permission for release of names or to introduce the study. Check with the IRB for further guidance.

Subjects will be recruited for this study by the Westat Corporation, which has recruited for studies at the U.S EPA HSF since 1998. The manner in which this will be done is similar that that of past U.S. EPA studies and specific recruitment procedures as per the previously UNC IRB-approved protocol, Recruitment and Screening of Potential Participants for U.S. EPA Studies (95-EPA-66). Every effort will be made to recruit women and members of racial minority groups into this study. Since this study will recruit older healthy subjects and one previous human study conducted at HSF before ("A pilot study to characterize cardiac biomakers in a healthy, 55-80 year-old population"; IRB #: 05-EPA-210; Graff, Pharm D) also involved the same age groups, therefore we are likely to re-contact with these subjects to check if they are interested in participation in this study. Subjects will be asked to call the recruitment office. During the telephone interview, the subjects will receive information regarding the study and their eligibility for the study will be assessed. Subjects who provide responses which indicate that they are likely to meet the criteria will be scheduled for an appointment in the Medical Station in the U.S. Human Studies Facility. At that time the study protocol will be outlined, and a medical history form will be administered and the completed one will be mailed to Westat as per 95-EPA-66.

B.2. **Protected Health Information (PHI).** If you need to access Protected Health Information (PHI) to identify potential subjects who will then be contacted, you will need a *limited waiver of HIPAA authorization.* If this applies to your study, please provide the following information.

a. Under this limited waiver, you are allowed to access and use only the minimum amount of PHI necessary to review eligibility criteria and contact potential subjects. What information are you planning to collect for this purpose?

b. How will confidentiality/privacy be protected prior to ascertaining desire to participate?

c. When and how will you destroy the contact information if an individual declines participation?

B.3. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable.** Include the number of required contacts and approximate duration of each contact.

It is anticipated that the duration of the pilot study will be approximately 3 months and the main study will take approximately 18 months. Participant recruitment and screening is expected to be continuous throughout the study until the intended number of participants is reached.

If the subject is eligible for the pilot study they will have 9 visits to the HSF over approximately 18-20 weeks. Subjects will have about 2 weeks break between 2 exposures. If the subject is eligible for the main study of diesel exhaust exposure the individual will have 5 visits to the research facility over approximately 5-6 weeks. The pre-enrollment genotyping and dietary assessment day visit will take approximately 1 hour. It may also be determined that the individual is not eligible for continuation in the study after genotyping of the blood sample. The physical exam will take approximately 2 hours. The training day (including fish oil/olive oil supplements assignment for the main study) will require approximately 3 hours. Exposure day will last approximately 8 hours for the pilot and main studies. Eighteen hours after the exposure, the subject will return for a follow-up visit which will last approximately 3 hours for the pilot and the main study.

B.4. **Where will the subjects be studied?** Describe locations where subjects will be studied, both on and off the UNC-CH campus.

Subjects will be seen in the U.S. EPA Human Studies Facility on Mason Farm Road in Chapel Hill, NC.

B.5. **Privacy.** Describe procedures that will ensure privacy of the subjects in this study. Examples include the setting for interviews, phone conversations, or physical examinations; communication methods or mailed materials (e.g., mailings should not indicate disease status or focus of study on the envelope).

All interviews, phone conversations, and physical examinations will be conducted in private rooms in the U.S. EPA Human Studies Facility. This facility is guarded and only individuals working in the building have access beyond the guard's desk without an escort. Additionally, subjects will need to initial the consent form indicating whether or not they would be willing to participate in the study with another volunteer present.

B.6. **Inducements for participation.** Describe all inducements to participate, monetary or non-monetary. If monetary, specify the amount and schedule for payments and if/how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it. For compensation in foreign currency, provide a US$ equivalent. Provide evidence that the amount is not coercive (e.g., describe purchasing power for foreign countries). Be aware that payment over a certain amount may require the collection of the subjects' Social Security Numbers. If a subject is paid more than $40.00 at one time or cumulatively more than $200.00 per year, collection of subjects' Social Security Number is required (University policy) using the Social Security Number collection consent addendum found under forms on the IRB website (look for Study Subject Reimbursement Form).

Subjects will receive monetary compensation for their time (approximately $12 per hour) and for procedures in the study. In addition, subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the US Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

A subject who is unable to complete the study for voluntary reasons or failure to comply with eligibility requirements will receive full compensation for his/her participation up to that point. Subjects who are dismissed by the investigators after enrollment in the study but prior to completion for involuntary reasons will be paid for the entire study, excluding completion bonus.

In the event a scheduled study activity must be cancelled by the investigators with less than 72 hours prior notice, the subject will be paid at the standard hourly rate for the time scheduled and canceled, and will be paid 50% up to a total maximum of $100 for canceled procedures. Cancellations could occur due to adverse weather conditions, equipment failure, or other unforeseen events. When feasible, the subject will be rescheduled.

The following table details the expected compensation for completion of the entire study: Subjects will be paid approximately $12 per hour for participation in this study. Total compensation will be based on the sessions that will be required of the individual in the study.

1. If following genotyping of the blood samples on screening day, it is determined that the individual's genotype is not needed for completion of the study, then that subject may be informed that his/her participation in this study is no longer necessary. The subject will be notified by phone and the total compensation for this study will be approximately $60.
2. If the subject is qualified and finished the entire pilot study, the total compensation for completion of this study will be approximate $2053.
3. If the subject is qualified and finished the entire main study, the total compensation for completion of this study will be approximate $1195.

Pre-study qualifications

Recruitment screening	$15
Physical exam	$15
Venipuncture (~50ml of pilot and ~80ml of main study; genotyping)	$30

Pre-study qualification total = $60

Subjects based on their genotype assessment may not be asked to further participate in the study.

Training day (3 hours)

Time (3h @$12/h)	$36
Holter monitor	$100

Pilot study:

Exposure (8 hours)

Venipuncture (~50ml, pre; 3@$30 each)	$90
Holter monitor (3 @$100 each)	$300
Chamber exposure (3 @$72 each)	$216
Venipuncture (~50ml, post; 3@$30 each)	$90
Time (3X8h @$12/h)	$228
Finger artery Endo-PAT (6 @$10 each)	$60
Brachial artery ultrasound (6@$50 each)	$300
On-time bonus (3@$25 each)	$75
Urine samplings (6 @$10 each)	$60

Total for completion of exposure = $1579

Eighteen hours after exposure (3 hour)

Venipuncture (~50ml; 3@$30 each)	$90
Time (3X3h @$12/h)	$108
Finger artery Endo-PAT (3 @$10 each)	$30

Total for completion of post-exposure = $228

Protocol Completion Bonus (3@$50 each)	$150

Approximate TOTAL for completion of pilot study = $2053

Main study:

Exposure (8 hours)

Venipuncture (~80ml, pre)	$30
Holter monitor	$100
Chamber exposure (2 hours)	$72
Venipuncture (~80ml, post)	$30
Time (8h @$12/h)	$96
Brachial artery ultrasound (2 @$50 each)	$100
Finger artery Endo-PAT (2 @$10 each)	$20
Food records (2 times)	$300
FFQ	$50
On-time bonus	$25

Total for completion of exposure = $823

Eighteen hours after exposure (3 hour)

Venipuncture (~80ml)	$30
Time (3h @$12/h)	$36
Finger artery Endo-PAT	$10

Total for completion of post-exposure = $76

Protocol Completion Bonus $100

Approximate TOTAL for completion of main study = $1195

Subjects will be provided a lunch by GCRC for the exposure day. If a subject is terminated from the study or chooses to withdraw he/she will be reimbursed for time and procedures completed up to that time point.

B.7. **Costs to be borne by subjects.** Include child care, travel, parking, clinic fees, diagnostic and laboratory studies, drugs, devices, all professional fees, etc. If there are no costs to subjects other than their time to participate, indicate this.

There will be no cost to the subject. Subjects traveling from areas beyond Chapel Hill/Carrboro will be reimbursed for travel expenses commensurate with the U.S. Government mileage rate in effect at the time. Parking will be provided or costs will be paid. Payments will be made after each segment of the study, unless the subject requests otherwise.

Part C. Questions for Studies using Data, Records or Human Biological Specimens without Direct Contact with Subjects

→ ***If this does not apply to your study, do not submit this section.***

C.1. What records, data or human biological specimens will you be using? *(check all that apply)*:

__ Data already collected for another research study
__ Data already collected for administrative purposes (e.g., Medicare data, hospital discharge data)
__ Medical records (custodian may also require form, e.g., HD-974 if UNC-Health Care System)
__ Electronic information from clinical database (custodian may also require form)
__ Patient specimens (tissues, blood, serum, surgical discards, etc.)
__ Other (specify):

C.2. For each of the boxes checked in 1, how were the original data, records, or human biological specimens collected? Describe the process of data collection including consent, if applicable.

C.3. For each of the boxes checked in 1, where do these data, records or human biological specimens currently reside?

C.4. For each of the boxes checked in 1, from whom do you have permission to use the data, records or human biological specimens? Include data use agreements, if required by the custodian of data that are not publicly available.

C.5. If the research involves human biological specimens, has the purpose for which they were collected been met before removal of any excess? For example, has the pathologist in charge or the clinical laboratory director certified that the original clinical purpose has been satisfied? Explain if necessary.

__ yes __ no __ not applicable (explain)

C.6. Do all of these data records or specimens exist at the time of this application? If not, explain how prospective data collection will occur.

__ yes __ no If no, explain

SUBJ #_____ DATE_____

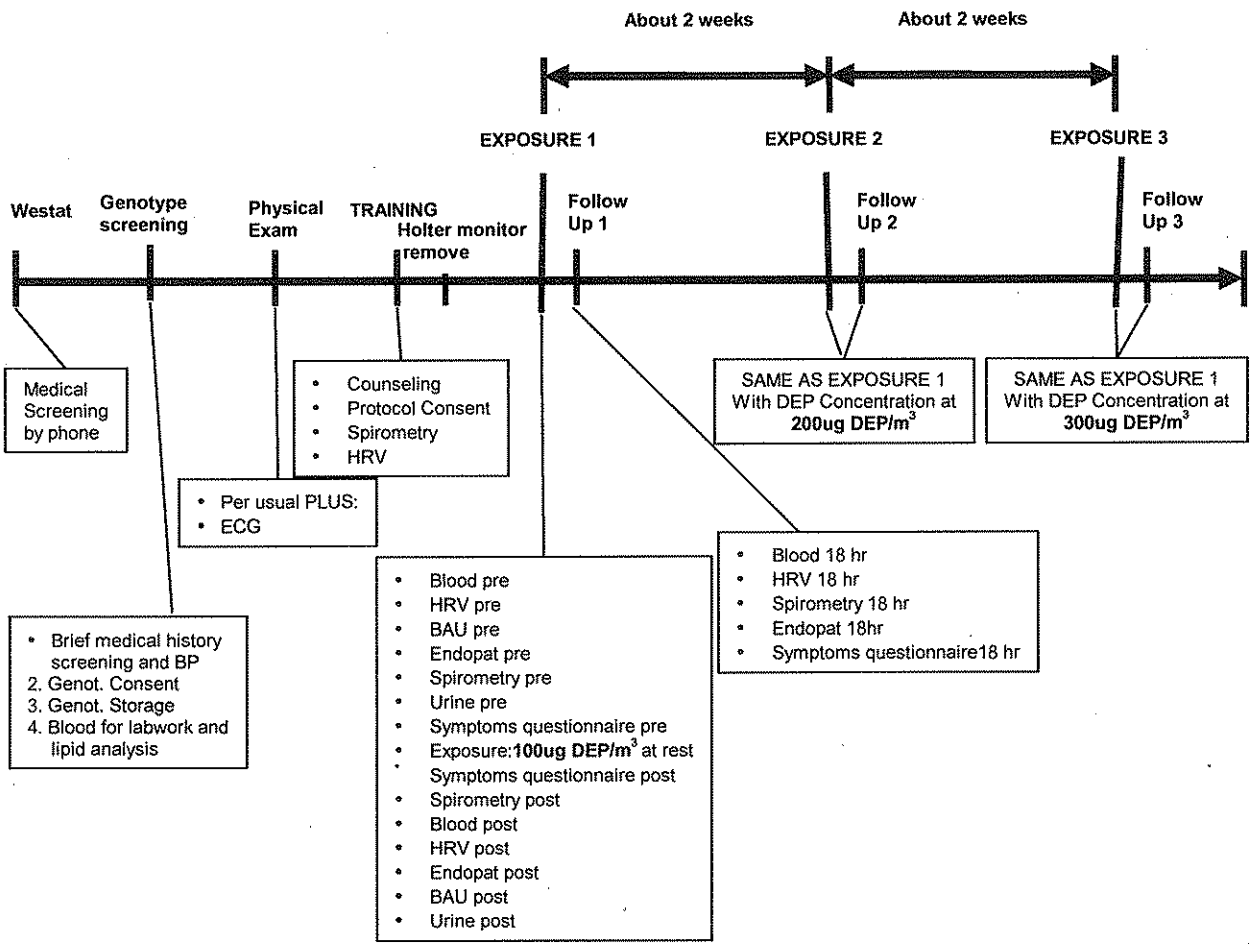
SYMPTOM QUESTIONNAIRE FOR CHAMBER EXPOSURE STUDIES

Pre-Exposure / End Exposure / 4 hrs post end Exposure (Circle one)

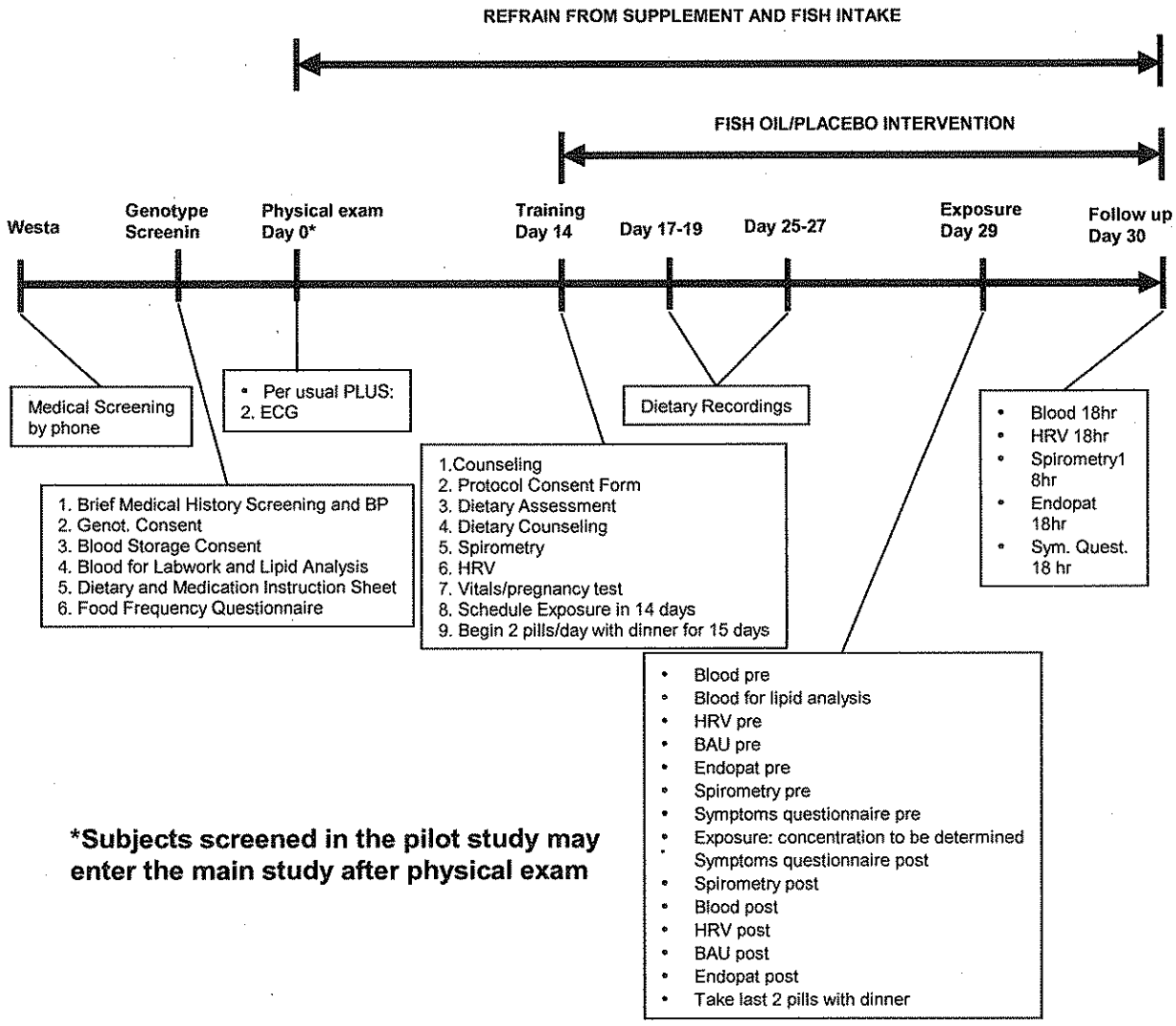
INSTRUCTIONS: Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = TRACE/NOTICED	(barely detectable)
2 = MILD/LIGHT	(present, but not annoying)
3 = MODERATE	(present, but somewhat annoying)
4 = SEVERE/HEAVY	(present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4



PILOT PHASE FLOW DIAGRAM



MAIN STUDY FLOW DIAGRAM